ANNEX 3

Synopsis of OECD Test Guidelines for Studies Included in the SIDS*

This document provides a summary of the information which should be reported for specific tests of the Screening Information Data Set (SIDS) as extracted from the appropriate OECD Test Guidelines. It is not intended to be inclusive or exhaustive; in cases of uncertainty over the interpretation of the acceptability of a study, evaluators should consult the relevant guideline or other guidance documents such as the following, or flag the issue for further discussion at the SIDS review process:

- Quality of and Access to Data Used to Prepare SIDS Dossiers (Section 3.2);
- Considerations for Developing SIDS Testing Plans (Section 3.3);
- Guidance for Meeting the SIDS Requirements (The SIDS Guide) (Section 3.4); and
- Considerations Concerning Adequacy of Data in the SIDS (Section 3.5).

Figures in [ ] correspond to the data elements in the HEDSET and the Revised OECD HPV Form 1.

* This document was prepared by the OECD Secretariat based on the agreements reached in the OECD Test Guideline and the Existing Chemicals Programme up to April 1996.

1. GENERAL INFORMATION

Each study should report full identity and the necessary references.

2. PHYSICAL-CHEMICAL DATA

General Considerations

Each study should report:

- full identification of substance tested (state, structural formula, spectra, purity, impurities stabilizers, etc. should be identified as completely as possible including percentage, potential impact where known, indicating sensitivity of tests in relation to impurities and their potential impact on health or the environment).

  Note: In SIDS work, tests for physical-chemical properties should be conducted in principle on the purified substance to avoid interference form impurities etc.

- methods: apparatus, procedure employed, sensitivity and accuracy of results, use of reference standards/controls, statistical analysis, any limitations or difficulties.

  Note: In SIDS work, when a standard test method such as OECD Test Guideline has been used, the method should be identified and it is not necessary to repeat details of the test in the HEDSET or in Revised OECD HPV Form 1.

- results: all results and all information relevant to interpretation of results.

Specific Consideratoins for Individual Data Elements

[2.1] MELTING POINT Ref. OECD TG 102

- m.p. or melting range expressed as °C;
- mean of at least 2 measurements ± range of accuracy of method;
- if decomposition occurs, the temperature of decomposition should be stated; for viscous liquids, " pour point" may be an acceptable alternative.

[2.2] BOILING POINT Ref. OECD TG 103

- mean of at least 2 measurements ± b.p. or b.p. range expressed as °C at a given pressure (KPa);
- measured values corrected to standard pressure;
- if substance decomposes before boiling, the temperature of decomposition should be stated.

[2.3] DENSITY Ref. OECD TG 109

- density expressed as kg/m^3, preferably at 20°C, as mean of at least 2 measurements;
- temperature at which the measurement was carried out, method of measurement in the Test Guideline, physical state of the substance should be reported.

[2.4] VAPOUR PRESSURE Ref. OECD TG 104

- v.p. should be determined for at least 3 temperatures in the range 0-50 °C, and a mean v.p. expressed as Pascal units (N/m^2) at 20 or 25 °C. This value should preferably be experimental but may be interpolated or extrapolated if necessary;
- transitions (change of state, decomposition) should be reported, giving temperature at which the change occurs at atmospheric pressure, and vapour pressure at 10 and 20 °C below and above transition.

[2.5] PARTITION COEFFICIENT N-OCTANOL/WATER Ref. OECD TG 107 and 117

- results expressed as log Pow at °C (measured or calculated, Annex of TG 117 can be used for calculation.)
- for TG 107, measurement concentrations in both phases for each determination (suggest 12 concentrations be reported (duplicate determinations of concentrations in n-octanol and in water under 3 different conditions)
- for TG 117, average retention data as mean of at least 2 measurements be reported in addition to details on fitted regression line, calibration methods, deadtime etc.
- any special considerations (e.g. if compound is surface active, dissociative, insoluble in water, metal organic)

[2.6.A.] WATER SOLUBILITY Ref. OECD TG 105

- results expressed as mg/l at °C, at least 2-5 replicates/trial, full test report should include calibration data for the chosen method and the readings for the test solutions;
- nature of carrier used for insoluble compounds;
- if the substance is insoluble in water the detection limits of the analytical method should be indicated.

[2.6.B.] PH VALUE AND PKA VALUE Ref. OECD TG 112

- For pKa, results expressed as pKa at °C, at least 3 replicates/trial.

- Full report should include all measured data and statistical parameters used for the calculation of pKa in addition to calibration data for the chosen method.

[2.12] OXIDATION - REDUCTION POTENTIAL

- OECD Test Guideline is not available for the measurement. However where applicable, indicate the redox potential and the conditions under which it was measured.

[2.13.A.] ADSORPTION/DESORPTION TO SOIL Ref. OECD TG 106

- Koc at °C, tests should be made for 3 soil types, at least 2 replicates/trial at one concentration in the screening test and at 4 concentration in the advanced test.

- Result of adsorption test, desorption test in the screening test, mass balance and adsorption isotherm data should be included in the full report.

3. ENVIRONMENTAL FATE AND PATHWAYS

[3.1] STABILITY

[3.1.1] PHOTODEGRADATION Ref. OECD TG 113

- For SIDS, estimation is sufficient. However, if a study is provided, methods used to measure degradation over time (14 days) should be discussed, including reference standards (if used), temperature, method of analysis, sensitivity, reproducibility etc.

- Results: changes observed on treated sample after testing; if possible, nature of decomposition products.

[3.1.2] STABILITY IN WATER (HYDROLYSIS) Ref. OECD TG 111

- should be examined at pH levels likely to be found in the environment (pH 4-9).

  Test Methods: should include information on identity and purity of test compound, reference standards, control, if used; detailed test procedures including test medium, temperature, pH,Buffer for each set of experiments, extraction method and recovery data if an extraction methods is used;

Results: should be expressed in terms of half life ($t_{½}$) in days, or for unstable compounds (in hours).

i) Initial Test - soluble substances, should be carried out at 50 °C for 5 days to indicate stability (solubility > 10^{-6} g/l) at a range of pH (4.0, 7.0, 9.0).

Results: if < 10% hydrolysis is seen after 5 days, ($t_{1/2}$ > 1 year) the product is considered hydrologically stable and no further testing is required. If the substance is unstable at environmentally relevant temperatures, e.g. if $t_{1/2}$ <1 day at 25 °C, no further testing is required.

ii) Further testing
For unstable compounds (1 year > $t_{1/2}$ > 1 day), further testing at 2-3 appropriate temperatures may be required.

[3.3] TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

- Methods for estimating environmental concentrations and fate are presented in "Guidance for Initial Assessment of Environmental Exposure", "Report of the OECD Workshop on the Application of Simple Models for Environmental Exposure Assessment (OECD Monograph No. 69)" and "Compendium of Environmental Exposure Assessment Methods for Chemicals" (OECD Environment Monograph No. 27). Diskettes of PC programme of models in the guidance document are available from the SIDS Contact Points or the Secretariat (see Section 4.3 of this Manual).

- Calculation of distribution by a Fugacity Level 1 type model should be provided.

[3.5] BIODEGRADATION

- Choice of testing protocols should be determined by physical-chemical properties (e.g.,solubility, volatility) and structure of test substances, e.g., - for soluble/insoluble substances tests based on dissolved organic carbon (DOC) or oxygen depletion and carbon dioxide generation. For volatile substances a closed system may need to be employed.

Methods:
- All methods should be reported including rationale, sensitivity and suitability for test substance; use of positive reference standards, (sterile) controls; calculation of degradation curve, statistical analysis, confidence limits, etc.;
- for poorly soluble test substances, the nature and concentration of any vehicles (solvents, processes) used to enhance the contact between test substance and microorganisms should be given.

Results:
- The biodegradation curve should be calculated and drawn, the experimental values showing a rate (%) of degradation over time (e.g. 28 days) should be given.
- If a reference standard is used, the biodegradation results and curve should be presented.
- Any inhibition of the degradation bacteria should be reported.
- Positive results: 60 % degradation (of theoretical maxima) in 28 days based on oxygen depletion or CO_2 generation tests, or 70 % for tests based on dissolved organic carbon may be regarded as biodegradable.

4. ECOTOXICITY

[4.1] ACUTE/PROLONGED TOXICITY TO FISH

ACUTE TOXICITY TO FISH Ref. OECD TG 203

Test Substance: complete identification including vehicles used to administer (e.g. solvents).

Test Species: numerous, see list of recommended species reference OECD Test Guideline 203

Methods: complete description of test procedure used (e.g. static, semi-static, flow through etc.) including water quality characteristics (pH, dissolved O_2 concentration, etc,) preparation of stock, test solutions, experimental procedures, etc.

- preferably should involve 5 concentrations of test solutions in geometric series, 10 fish/test group and control.

Results:

- maximum concentration causing no mortality in test period (preferably 96hr.);
- minimum concentration causing 100% mortality;
- cumulative mortality at each concentration and in controls;
- LC_{50} or EC_{50} at 24, 48, 72, and 96 hours based preferably on actual, not nominal, concentrations (with 95 per cent confidence limit at 96hr);
- statistical procedures used for determining the LC_{50} values;
- any incident that might affect results;
- abnormal responses of fish;
- % loss of concentration over the experiment, especially for poorly soluble substances (may affect calculation of LC_{50}s).

[4.2] ACUTE TOXICITY TO AQUATIC INVERTEBRATES

A. *DAPHNIA* Ref. OECD TG 202

ACUTE IMMOBILIZATION TEST

Test Substance: complete identification, including vehicles used to administer (e.g., solvents).

Test Organism: *Daphnia* sp. identified by species strain, source.

Methods:

- complete description including animal maintenance and housing, preparation of test and control solutions, physical regime, experimental procedure including number of replicates;
- at lease 20 Daphnia, not more than 24hrs old, should be used in groups of 5 each, tested at concentrations in a geometric series, in which highest concentration causes 100% immobilisation, and lowest should give no observable effect.

Results:

- number and % of Daphnia showing effects at 24 and 48 hr at each concentration and controls, nature of effect (immobilisation, mortality);
- the 24h and 48h EC_{50}, preferably with 95 per cent confidence limits, determined by a suitable method;
- if possible, the slope of the concentration response curve with its 95 per cent confidence limits;
- the highest concentration tested producing no immobile *Daphnia*;
- the lowest tested concentration producing 100 per cent immobile *Daphnia*

[4.3] TOXICITY TO AQUATIC PLANTS e.g. ALGAE

(Alga Growth Inhibition Test) Ref. OECD TG 201

Test Species: various species of fast growing green algae such as recognised strains of *Selenastrum capricornutm*, *Scenedesmus subspicatus*, *Chlorella vulgaris*.

Test Substance: full chemical description including vehicles used to administer (e.g. solvents).

Methods:

- complete housing, maintenance and test procedures and conditions;
- install cell concentration;

- 5 graded concentrations tested, preferably 3 replicates, with highest dose inhibiting growth by 50-100 %, lowest dose at no observed effect relative to controls.

Results:
- calculation of algal growth over test period (3 days) - e.g. mean cell concentration at 24, 48, 72 hours for each time and dose, plotted to give growth curves;
- EC_{50} and method of calculation;
- NOEC;
- any observed adverse or unusual effects.

[4.5] CHRONIC TOXICITY TO AQUATIC ORGANISM

[4.5.2] CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

DAPHNIA Ref. OECD TG 202

PROLONGED TOXICITY TO DAPHNIDS/REPRODUCTION TEST

Test Substance: complete identification, including vehicles used to administer (e.g., solvents).

Test Organism: *Daphnia* sp. identified by species strain, source.

Methods:
- complete description (see [4.2.] A.);
- 5 concentrations in geometric series of test solution, highest at 24 hr EC_{50}, lowest at 1/100 24 hr EC_{50};
- at least 40 animals, 4 groups of 10, at each concentration,: controls
- test duration 14-21 days or until at least 3 broods of F1 have appeared in controls.

Results:
- The EC_{50} (immobilisation) and EC_{50} (reproduction) values as far as possible at 24, 48, 96 hrs, 7 days, 14 days, and at the end of the test: statistical analysis and 95 per cent confidence limits;
- the length of time for the appearance of the first brood for each concentration;
- the number of young alive in each test vessel at any given day at which counts were made (the minimum requirement is for counts thrice weekly);
- the number of dead young on each day of counting;
- the highest concentration tested at which no significant difference is found versus the controls with respect to mortality, reproduction and other observed effects;
- the lowest concentration tested with significant difference versus the controls.

[4.6] TOXICITY TO TERRESTRIAL ORGANISM

- required if significant exposure is expected in the terrestrial environment.

[4.6.1] TOXICITY TO SOIL DWELLING ORGANISM Ref. OECD TG 207

Test Species: *Eisenia foetida*, adults, mean weight 300-600 mg.

Test substance: Full chemical description.

Methods:
- (artificial) soil tests are preferred to contact filter paper tests as being more representative of the natural habitat;
  1) Artificial Soil Test - 10 worms/vial artificial soil;
  2) Filter Test 10-20 worms/test, housed separately,
- range finding test may precede dose testing;
- testing at 5 geometric dose concentrations plus control, reference standard;

- assessment of mortality at 7, 14 days.

Results should indicate:
- reports of all test methods, procedures, etc.;
- calculation of LC_0, LC_{50}, LC_{100};
- dose response curve;
- statistical analysis and confidence levels;
- any observed adverse or unusual effects.

[4.6.2] TOXICITY TO TERRESTRIAL PLANTS Ref. OECD TG 208

Test Species: minimum of 3 species from different plant types, variety, seed source.

Test Substance: complete identification.

Route of Administration: soil, incorporation into seed bed.

Test Conditions: control, plus 3 concentrations tested in random block design, minimum 4 replicates/treatment; 5 seed/replicate.

Methods: complete description of test conditions, including growth conditions, soil characteristics, etc.

Results: should give:
- concentration-effect curve;
- LC_{50} for emergence; EC_{50} for growth;
- phytotoxic effects for treatments, control, etc.;
- statistical analysis of results, if possible.

[4.6.3] TOXICITY TO OTHER NON-MAMMALIAN TERRESTRIAL SPECIES (INCLUDING AVIANS)

A. AVIAN DIETARY TOXICITY TEST Ref. OECD TG 205

Test Species: one or more species, (mallard duck, Japanese or bobwhite quail, pigeon, ring-necked pheasant, red-legged partridge) from healthy known stock, 10-17 days old.

Route of Administration: Oral in diet.

Housing Regimes: consistent with good laboratory and humane practice, details of housing regime, diet, preparation/maintenance, including test diets, etc..

Testing:
- One dose level testing
  If 5,000 ppm in diet produces no toxicity within 8 days, then further testing may be discontinued.

- Multiple dose testing
  5 graduated dose levels, 2 controls and 1 treatment group/dose, 10 birds/group for 5 days followed by 3 days of no treatment; reference substances (optional).

Reporting of Results
- frequency, duration of observations;
- number of deaths/treatment group and /controls;
- average body weights at beginning, end of exposure period, end of test;
- observations on intoxications/behavioural changes including food consumptions;
- LC_0, LC_{50}, LC_{100} values, confidence limits and statistical analysis.

B. AVIAN REPRODUCTION TEST Ref. OECD TG 206

Test Species:one or more species, usually mallard, bob white or Japanese quail of healthy individuals from the same population, known parentage.

Test Substance: complete chemical identification.

Route of Administration: Oral in diet, 20 weeks.

Methods:

i) Housing regime: complete description of housing conditions including diet/supplements/carriers, frequency of feeding, clearing, etc., procedures on rearing, acclimatization, methods of identification of birds, eggs, conditions of egg storage.

ii) Test conditions: complete description including dosing levels, frequency, use of controls, use of positive reference standards (optional)

- should test at 3 dietary concentrations, highest dose at 50% LC_{10} from acute testing, others at lower levels, lowest dose no observed effect level.

Results should give:
- the number of deaths at each treatment level, and in controls;
- all weight changes in birds over test period (beginning to end of exposure period and at end of tests); mean weight change;
- frequency, duration, description of toxicity on adult and young birds;
- gross pathological results, residue analysis (if performed);
- egg production, egg set, viability, hatchability (including normal hatchlings), survival of young, and eggshell thickness (in summary by concentration level and for each group by week for the test period);
- no effect level, and any statistically significant effect levels;
- any observations about unusual effects.

5. TOXICITY

[5.0] GENERAL CONSIDERATIONS

Common general considerations which should be reported for each toxicology study are:

Test Substance:
- full chemical identification, CAS number etc.;

- full identification of physical characteristics (including: state, purity, identification of impurities, solubility, stability, solvent/vehicles used in experiment).

Test Species:
- adequate numbers of healthy animals should be used;
- individuals in test groups should not vary more than +- 20% of mean weight of the group;
- females should be virgin;
- common laboratory strains should be used wherever possible.

Housing Conditions should be reported indicating housing conditions, routine, consistent care, conventional laboratory diets, unlimited water, consistent conditions of temperature, light, relative humidity etc.

Clinical Examinations should be frequent enough to ensure observation of critical signs but at least once a day; should include records of all deaths, behavioural changes, necropsy results.

[5.1] ACUTE TOXICITY Ref. OECD TG 401-3, 420

Test Species:

- oral: rat (preferred);
- inhalation: rat (preferred) or rabbit;
- dermal: rat, rabbit, guinea pig (other species may be considered). Commonly used strains, weight variation of each animal should not exceed +- 20% mean weight.

Route of Exposure: oral, dermal (liquids, solids), inhalation (gases, vapours, fine powders), as appropriate.

Number/Sex of Test Animals:
- for first test (oral, dermal) at least 5 healthy young animals/dose level/all same sex;
- for inhalation - 10 animals (5 of each sex);
- females should be virgin.

Method:
- a limit test may be carried out at one dose level (2000 mg/kg body weight) on groups of 5 males or 5 females for oral or dermal. For inhalation, 10 animals should be tested; if compound-related mortality is observed, a full study may be needed;
- dose levels: oral, dermal: at least 3, plus controls with a range of toxic effects and mortality rates so that dose response curve can be prepared and an LD_{50} can be determined; inhalation: if one continuous dose, 4 hr exposure produces no mortality at 5 mg/L, then further tests may be discontinued. If effects, a full study with 4-hours exposure at 3 dose-levels may be required;
- animals observed for 14 days with clinical observations daily, including weight change, pathological examinations reported upon termination of animals.

Results:
- LD_{50} value given for each sex, or a combined LD_{50} if both sexes are tested. Any significant difference in response between sexes (>2-fold) should be reported;
- dose mortality curve, confidence limits;
- report all signs of toxicity (immediate or delayed), number dead, number with no effect for all dose levels, control;
- necropsy and histopathological effects should be reported for those due to test substance;
- for dermal: effects (local) at site of application should be reported;
- for inhalation study: (range of) particle size of test substance should be reported.

[5.4] REPEATED DOSE STUDY (14- 28 DAY) Ref. OECD TG 407, 410, 412

Test Species:
- oral, inhalation: rat preferred, 6-8 weeks old;
- dermal: rat, rabbit, guinea pig.

Route of exposure: Oral preferred, diet, gavage. For dermal or inhalation reasons should be given.

Number and sex of test animals:
- 10/dose level (5 of each sex);
- more, if interim sacrifices.

Dose levels:

- limit test: 1 dose ≥ 1000 mg/kg. If no observable effects or evidence of toxicity at this level, then a full study may not be required.

- full test: 3 doses plus control
  • lowest - no evidence of toxicity
  • middle - minimal observable toxic effects
  • highest - toxic effects noted, but fatalities do not affect evaluation of results.

Methods:
- all information regarding housing, care of animals, methods used, experimental procedures should be reported;
- for the inhalation study, air flow rates, concentrations, particle size distribution, etc. should also be reported.

Report:
- clinical observations on animal behaviour, weight change, etc., laboratory findings on the number of dead, sick, affected animals by sex, dose duration of exposure for entire experimental period;
- time of death or effects during, after exposure related to exposure level, time of onset of symptoms, duration and recovery, if any;
- if no signs of toxicity seen, this should also be reported;
- results of necropsy (all lesions, abnormalities, organ weight changes) should be reported related to dose level and as a deviation from controls; haematological tests, clinical biochemistry, histopathology should all be reported to controls.

Note: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (OECD Test Guideline 421) is acceptable.

[5.5] and [5.6] GENETIC TOXICITY (*in vitro* and *in vivo*)

A range of *in vivo* and *in vitro* studies are acceptable. Testing should be sufficient to cover two different endpoints:

(1) gene mutation (usually with bacteria)
(2) chromosomal aberrations/changes (usually non-bacterial).

Although many mutagenicity tests are available, approximately five are commonly used; one, the *Salmonella* mutation assays is virtually globally routine. Two to five tests have been suggested as reasonable, in general, the *Salmonella* reverse mutation assay in combination with test(s) for induction of chromosome aberrations.

Selection of test methods should take into account the nature of the test material, data from other toxicological studies, sensitivity of strains chosen, the potential for false positives or negatives, the use of reference substances as positive controls, response with and without metabolic activation, merits of *in vitro* vs. *in vivo*, eukaryotic vs. prokaryotic testing, etc.

Chromosomal aberrations and changes can be determined by a series of non-bacterial tests *in vitro* or *in vivo*.

[5.5.A.] BACTERIAL TEST Ref. OECD TG 471 and 472

Measures: Gene mutations
Cell cultures: *Salmonella typhimurim; Escherichia coli*

Methods:
- full identification of bacteria and strains used, positive reference substances and negative controls;
- details of test methods chosen and procedures followed (culture conditions, dose levels, rationale for dose selection, number of plates per test, metabolic activations system chosen, media composition; number of replicates);

Results:
- individual plate counts of strains used, mean number of revertant colonies/plate;
- report details of response (magnitude of increased revertants, plus any dose response) in relation to controls;
- state whether overall result is negative or positive for experiments both with/without metabolic activation;
- if negative results obtained, then justification of dose levels used, and type of metabolizing system in relation to the structure of the test organisms, and whether positive controls gave satisfactory response, should be reported;
- statistical analysis with standard deviation.

[5.5.B.] NON-BACTERIAL *IN VITRO* TEST

i) MAMMALIAN CELL GENE MUTATION TEST Ref. OECD TG 476

Measures: base pair mutations/small deletions.

Cell cultures: any appropriate mammalian cell culture, e.g. L5178Y mouse lymphoma cells, CHO, V-79 Chinese hamster cells.

Methods:
- test should be designated to have a predetermined sensitivity and power. No cells/culture is usually 1/10 expected background mutant frequency; usually 4 concentrations of test substance to yield concentration - related toxic effect;
- highest concentration should produce a low level of survival;
- positive and negative controls should be tested;
- cells should be exposed to test both with and without metabolic activation over a (1-5 hr) suitable exposure period.

Report: see also [5.5.A.]
- details of methods used (e.g. individual colony counts/treated and control groups survival and cloning efficiencies (% of controls); mutant frequency (number of mutants/no surviving cells) selective agents;
- methods used to enumerate numbers of viable and mutant cells;
- dose-response relationship where possible;
- statistical analysis and confidence limits where possible.

Results: as per [5.5.A.]

ii) *IN VITRO* MAMMALIAN CYTOGENETIC TEST (CHROMOSOMAL ABBERATIONS / CHANGES)
Ref. OECD TG 473

Culture: a variety of cells lines, strains or primary cultures can be used.

Method: should describe the test conditions, including cell culture technique, exposure in both presence and absence of appropriate metabolic activation system, exposure period to test substance, exposure concentrations, test performance.

Report:
- type of test, time of fixation, number of replicates, use of positive reference substances,controls;
- the type and number of aberrations and method of scoring, for each treated and control culture;
- details of response - e.g., type of structural damage, magnitude of effect, any dose response, statistical analysis, if done;
- state whether overall effect is positive or negative for experiments both with and without metabolic activation.

If there are data on the effect of the chemical on cell cycle time, the adequacy of the selected exposure periods should be indicated.

[5.6] GENETIC TOXICITY *in vivo*

i) SEX-LINKED RECESSIVE LETHAL TEST *DROSOPHILA* (GENE MUTATION)
Ref. OECD TG 477

Measures: Point mutations and small deletions in germ line (X chromosome) of insect by sex linked recessive lethal

Species: Wild type males and females of Muller 5 stock or appropriate strain with inverted X chromosomes.

Routes of exposure: oral, injection of inhalation.

Method:
- report all procedures followed, test conditions;
- dose: maximum tolerated concentration, or one producing some indications of toxicity;
- as positive reference control plus blank control.

Results:
- report number of lethal mutations/dose, statistical analysis;
- state whether overall result is positive or negative. If negative, justify that dose levels, methods are adequate (by use structure/reactivity of the test substance).

ii) NON-BACTERIAL *IN VIVO* TESTS (CHROMOSOMAL ABERRATIONS/CHANGES)
Ref. OECD TG 474, 475

MICRONUCLEUS TEST
MAMMALIAN BONE MARROW CYTOGENETIC TEST

Species: any appropriate mammal, usually mouse, but also rat, hamster.

Route of Exposure: oral, intraperitoneal injection or other appropriate route.

Sex: young healthy animals, usually 5/sex, 10 animals /group, treatment and control groups.

Treatment regime: should be reported including description of test conditions.

Dose:
a) single administration of maximum tolerated dose or that producing indication of cytotoxicity.
b) repeated dose, sample to be taken 12 hrs after dosing at intervals to 72 hrs.

Test size:
- should score approximately 1000 micronucleated polychromatic erythrocytes/animal (MPE's).

Results:
- test report should include information on the animals used treatment, etc. details of test conditions, criteria used to identified MPE's;
- a positive result should be indicated by:
  i) a statistically significant dose-related increase in number of micronucleated polychromatic erythrocytes;
  ii) detection of a reproducible and statistically significant positive response at a test point;
- a negative result should be checked to ensure test substance is reaching target tissue, to confirm value of test as a mutagenicity screen;
- additional mutagenicity tests, involving different parameters, e.g. chromatid exchange, crossover, forward and reverse mutations etc. are described in the OECD Test Guidelines.

[5.8] TOXICITY TO REPRODUCTION

Ref. (1) One Generation Reproductions Toxicity Study OECD TG 415
(2) Two Generation Reproduction Toxicity Study OECD TG 416

Test species: Rat or mouse.
Route of Exposure: usually oral, daily from 5-9 wks age.
Number of animals/sex:
- enough animals to give 20 pregnant females to term if possible;
- males should be dosed for at least one complete spermatogenic cycle (56 days mouse; 70 days rat);
- p generation females should be dosed for two oestrous cycles, then observed throughout the entire cycle.

Dose levels and exposure: 3 treatment groups plus control; highest level should induce toxicity but not mortality medium level should induce minimal toxic effects, lowest group no observable adverse effects.

Methods:
- one high dose > 1000 mg/kg - if no evidence of effects further studies may not be required;
- daily observation noting any changes or effects.

Results:
should include:
- species, strain used;
- toxic response data by sex and dose, including fertility, gestation, and viability indices;
- time of death during the study or whether animals survived to termination;
- table presenting the weights of each litter, the mean pup weights and the individual weights of the pups at termination;
- toxic or other effects on reproduction, offsprings, postnatal growth, etc.;
- the data of observation of each abnormal sign and its subsequent course;
- body weight data for p generation animals;
- necropsy findings, a detailed description of gross necroscopy and histopathology, of all reproductive organs;
- statistical analysis of results;
- no effect level and adverse effects on reproduction, parturition, lactation and post natal growth.

Note: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test (OECD Test Guideline 422) and Reproduction/Developmental Toxicity Screening Test (OECD Test Guideline 421) are also acceptable.